

## ***Conolophus marthae* sp.nov. (Squamata, Iguanidae), a new species of land iguana from the Galápagos archipelago**

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### **Abstract**

*Conolophus marthae* sp. nov., a new species endemic to Volcan Wolf of northern Isla Isabela of the Galápagos archipelago, is described. The new species is morphologically, behaviorally, and genetically distinguished from the other two congeneric species *C. subcristatus* and *C. pallidus*. Besides the taxonomic implications, *C. marthae* sp. nov. is extremely important as it is the only evidence of deep divergence within the Galápagos land iguana lineage.

**Key words:** Galápagos pink land iguana, *Conolophus*, Iguanidae, Squamata, Galápagos Islands, Galápagos National Park, lizards, endemism

### **Introduction**

Land iguanas from the Galápagos are among the most emblematic organisms of that archipelago. The current distribution of these reptiles reflects direct and indirect human impacts (Snell *et al.* 1984). Consequently, at present, land iguanas occur only in limited areas of a few islands. Current taxonomy of Galápagos land iguanas recognizes two species: *C. pallidus* Heller, 1903 and *C. subcristatus* (Gray, 1831). The first species occurs only on Santa Fe, whereas *C. subcristatus* occurs on Fernandina, Isabela, Santa Cruz, Plaza Sur, Seymour Norte (a translocated population), and Baltra (a repatriated population). Morphological (Snell *et al.* 1984) and genetic data (Rassmann *et al.* 2004; Tzika *et al.* 2008; Gentile *et al.* 2009) suggest that some populations of *C. subcristatus* may warrant specific status. Thus, the taxonomy of Galápagos land iguanas is incomplete and this may represent a further threat to the persistence of all species in the group (Daugherty *et al.* 1990).

Recently, a previously overlooked pink and black-striped species of land iguana was found in Galápagos Islands (Gentile *et al.* 2009). For its genetic, morphological, and behavioral characteristics, Gentile and collaborators identified this form as a distinct species. Despite recognition as distinct, the species remained unnamed. In fact, in previous works (Tzika *et al.* 2008; Gentile *et al.* 2009), it was referred to the pink form by using the term “rosada” (pink in Spanish), but such a term was disclaimed for nomenclature purposes (Gentile *et al.* 2009). Here, we name this new species and provide its formal description and diagnostic characteristics.

### **Materials and methods**

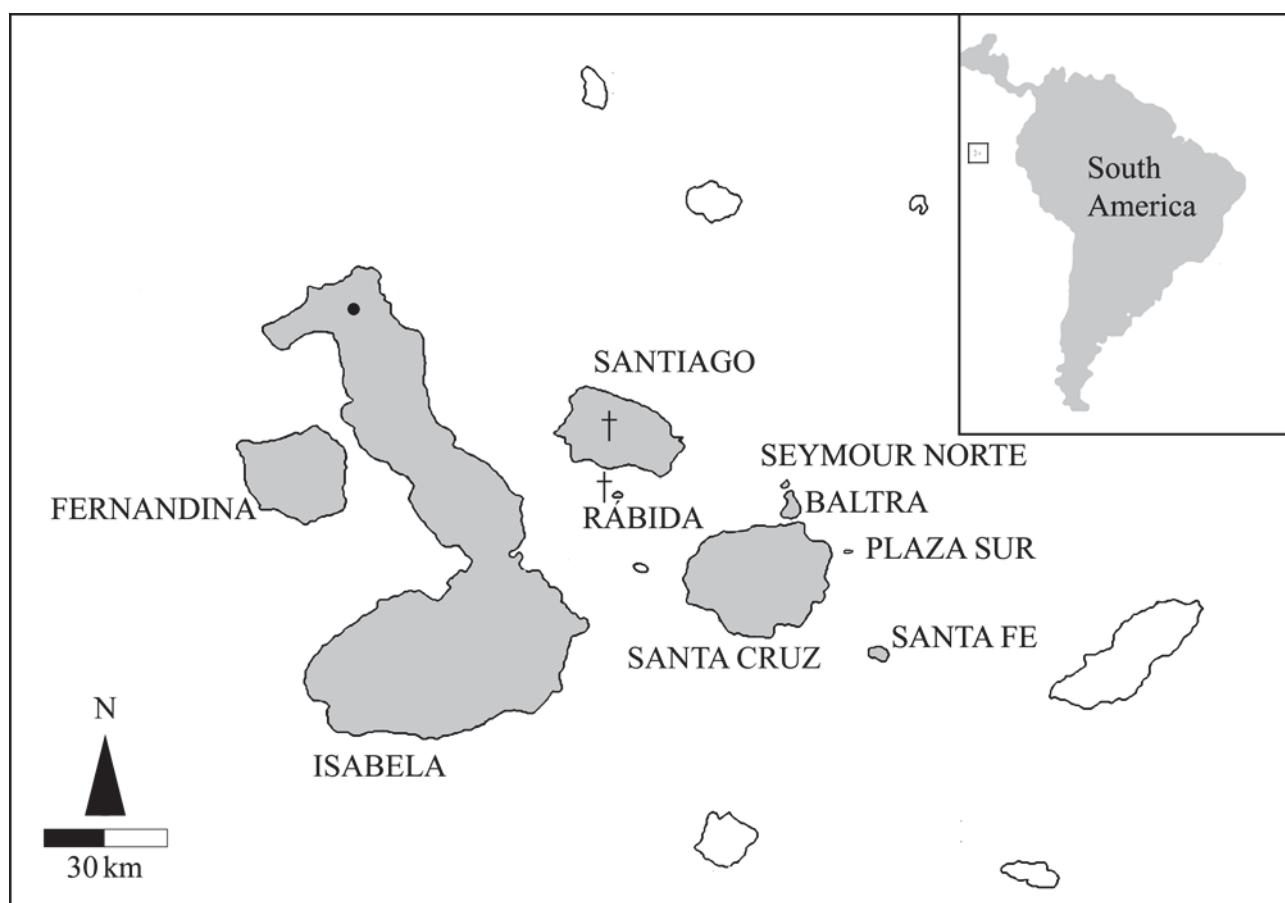
Morphological, behavioral, mitochondrial and nuclear DNA data are used to describe the new species and to distinguish it from other species of the genus *Conolophus*.

Snout-vent length (SVL) and vent to tip of the tail length (VTL) were measured to the nearest 0.1 cm, at

the maximum horizontal stretch of the body. Weight was measured with a dynamometer at the nearest 0.1 Kg. Head measurements were taken to the nearest 0.01 mm with digital calipers. Scale counts recorded per Snell *et al.* (1984). All measurements were taken *in vivo*.

Head-bob (nodding) behavior was recorded by a Panasonic NV-GS200 digital MiniDV camcorder. The head-bob display was investigated by analyzing the head movement in a 5x5 pixel square grid, frame by frame. Time interval between contiguous frames was 0.04 seconds. We used the software Imageready ver.9 (Adobe System Incorporated, San Jose, California). Although the frequency of bobs estimated by this method is correct, peak height may suffer from some inaccuracy due to the lack of standardized conditions under which movies were recorded. However, such possible inaccuracy does not affect the general pattern.

Genetic data were produced and analyzed per Gentile *et al.* (2009).



**FIGURE 1.** Galápagos archipelago. Grey indicates islands where land iguana species occur or have occurred in historic times. Crosses indicate extinction in whole island. A dot indicates Volcan Wolf.

#### *Conolophus marthae* new species

Galápagos pink land iguana

Figs. 2–5

**Holotype.** A free-ranging adult male permanently branded with the number 117. A Passive Integrated Transponder (PIT) with the number 091-601-303 was hypodermically inserted in one of the posterior legs. The individual was captured and released approximately four km north of the Equator on the top of Volcan Wolf, Isla Isabela, Galápagos National Park, Ecuador (0.03792° N; 91.36324° W, datum WGS84, as recorded by a Garmin 12CX handheld GPS). The individual was captured by A. Jaramillo on June 8<sup>th</sup> 2006, blood was drawn by G. Gentile. Photos were shot by G. Gentile. Blood in lysis buffer voucher n. MCZRR450 (as

reported in the Genbank records FJ716129 and FJ716130) is hosted in the reptile collection (as specimen n. R450) of the Civic Museum of Zoology (MCZR, Rome, Italy,). Original photo files, named as “Morphobank\_m27772.jpg” (Figure 2), “Morphobank\_m27773.jpg, Morphobank\_m27774.jpg, Morphobank\_m27775.jpg, Morphobank\_m27776.jpg, Morphobank\_m27777.jpg, and Morphobank\_m27778.jpg” (Figures 3A, 3B, 3C, 3D, 3E, and 3F, respectively), and the movie “Morphobank\_m27779.wmv” are included in a project titled as the present paper, hosted in Morphobank (<http://www.morphobank.org>). Such photos and video form a basis of the description and should be considered also as illustrating the type specimen, for purposes of Article 73.1.4 of the Code (ICZN, 1999), but see also the paragraph “Notes added in proofs”. All material refers to the same individual (free ranging, with PIT number 091-601-303), elected as Holotype.



**FIGURE 2.** *Conolophus marthae* sp. nov. Holotype. Adult male with brand number 117 and PIT number 091-601-303. The individual is changing skin.

**Diagnosis.** *Conolophus marthae* sp. nov. is distinguished from *C. pallidus* and *C. subcristatus* by the following color pattern: pinkish head, pinkish and black (dark) body and legs, with a typical black-striped pattern on the mid to posterior dorsal body; stripes are along the dorsal-ventral axis, may be irregular and their number variable; stripes may join to form a more complex pattern; stripes occur on the ventral body, but are less evident; dark tail.

Other distinctive, but slightly variable morphological traits co-occur in males: i) adipose nuchal crest with small or reduced conic scales, ii) poorly elevated (pyramid-shaped) or almost flat dorsal head scales.

*Conolophus marthae* sp. nov. is also distinguished from the other two congeneric species by a distinctive pattern of head-bob behavior (Fig. 6, see Morphobank accession code: p241).

The new species is unequivocally distinguished from *C. pallidus* and *C. subcristatus* by the several diagnostic sites in the sequence of the control region and cytochrome *b* gene of the mtDNA, reported in Table 1, and by a completely different, non overlapping, size-range of alleles at the microsatellite locus CS7 (Tzika *et al.*, 2008; Gentile *et al.* 2009). Alleles at locus CS7 range between 245 and 333 bases (as defined in Gentile *et al.* 2009).

#### Description of Holotype in life.

**Sex:** Male

**Age:** Adult

**Weight:** 5.0 Kg.

**Morphological measurements:** SVL: 47.0 cm; VTL: 61.4 cm; head length: 78.22 mm; head width: 63.76 mm; internostri distance: 17.89 mm; eye-eye distance: 35.19 mm.

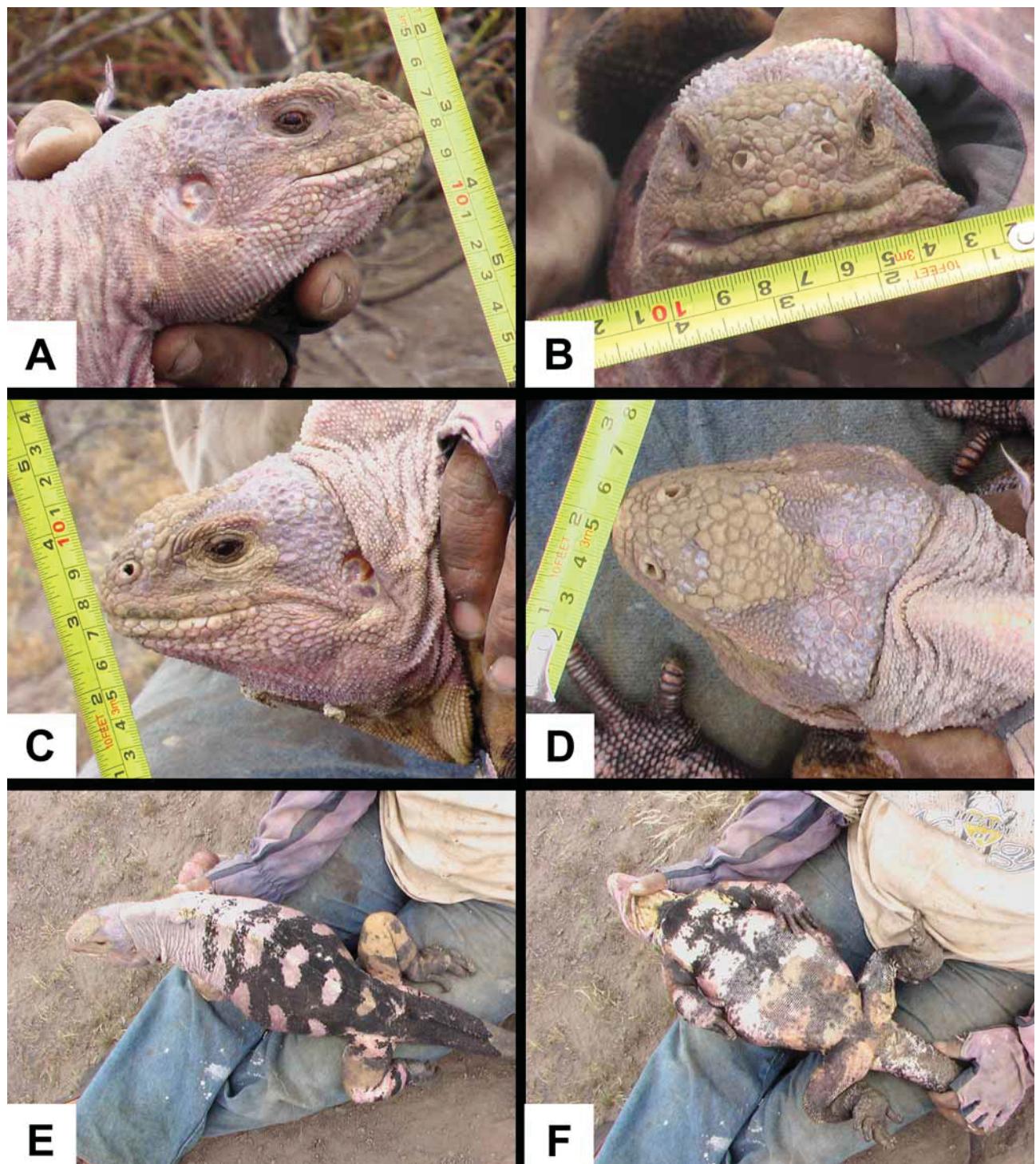
**TABLE 1.** Diagnostic sites in the sequence of the control region and cytochrome *b* gene in the mtDNA. Position are based on the Genbank records FJ716129 (Control region) and FJ716130 (cytochrome *b*). The sites 471 and 474 of the cytochrome *b* sequence are polymorphic in *C. subcristatus*, but still diagnostic.

		Control region																							
		24	25	63	85	92	93	167	168	179	198	204	205	247	318	335	508	509	512	651	696	834	850	1098	
<i>C. subcristatus</i> and <i>C. pallidus</i>		G	T	T	A	C	T	C	T	T	A	T	C	T	G	C	A	G	A	C	G	G	T	C	T
<i>C. marthae</i> sp. nov.		C	C	A	T	T	G	T	C	A	G	C	T	A	A	A	G	A	C	T	A	A	C	T	C
		Cytochrome <i>b</i>																							
		12	51	69	75	117	135	147	171	207	249	255	267	291	295	315	321	363	369	372	408	411	426	463	
<i>C. subcristatus</i>		C	C	C	C	C	C	C	C	C	A	T	T	T	C	A	A	A	G	C	C	T	G		
<i>C. pallidus</i>		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
<i>C. marthae</i> sp. nov.		T	T	T	T	T	T	T	T	A	T	C	C	C	T	G	T	T	A	T	T	C	T	A	
		465																							747
<i>C. subcristatus</i>		C	Y	R	T	C	C	C	T	G	A	C	C	T	C	T	A	A	G	T	T	G	A		
<i>C. pallidus</i>		.	T	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
<i>C. marthae</i> sp. nov.		T	G	C	C	T	T	T	C	A	G	A	T	C	T	C	A	C	G	G	C	C	A	C	
		786																							1087
<i>C. subcristatus</i>		A	T	T	C	C	C	T	C	C	T	C	T	C	T	G	C	A	T	A	G	C			
<i>C. pallidus</i>		.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
<i>C. marthae</i> sp. nov.		G	C	C	T	T	T	C	T	T	C	T	C	T	G	C	A	T	A	T	G	C	T	A	

**Meristic characteristics:** N. supralabial scales: 7 (left side) and 9 (right side); n. infralabial scales: 10 (left side) and 9 (right side); n. scales around the parietal scale: 8; n. scales around the mental scale: 9; n. scales around the rostral scale: 8; n. scales along the middle-dorsal line: 17; n. scales around the inguinal scar: 46. Number of femoral pores: 19 (left leg) and 18 (right leg).

**Morphological characteristics:** Snout elongated, not shortened. Tympanum taller than wide. Scales flat or almost flat above the tympanum, in the post-orbital region. Slightly more elevated pyramid-shaped scales occur in the dorsal head.

Nuchal crest pronounced, adipose, with small conic scales which are reduced or almost flat along the ridge of the anterior half of the crest. Conic scales are more prominent, but not spinose, along the ridge of the posterior half. Dorsal crest less developed, with small conic scales along the ridge. Caudal crest poorly developed. Round-cross-section tail, not laterally compressed. Fingers of fore and hind legs with short claws, not recurved.



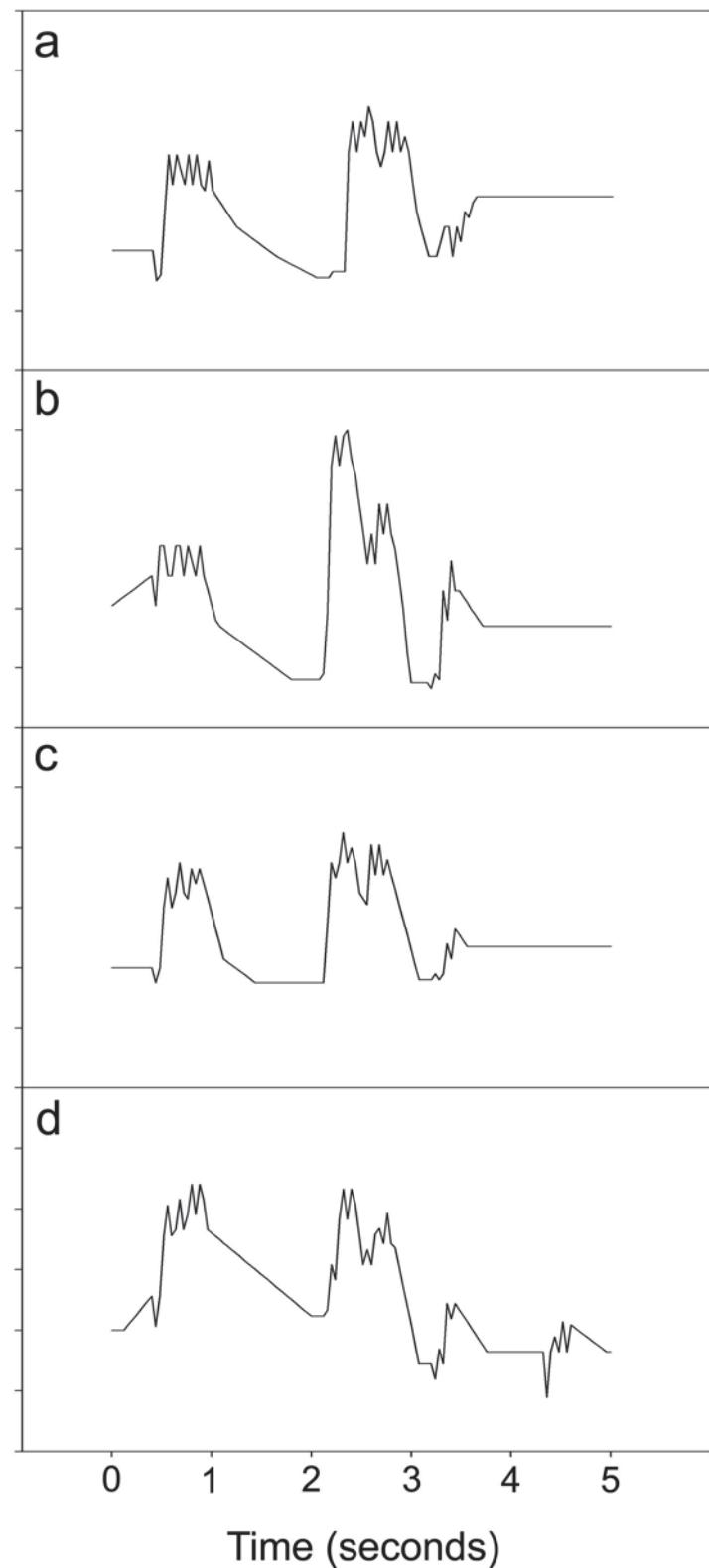
**FIGURE 3.** *Conolophus marthae* sp. nov. Holotype. Adult male with brand number 117 and PIT number 091-601-303. A–D) views of the head: right, frontal, left, and from above, respectively; E) dorsal view; F) ventral view. The individual is changing skin.



**FIGURE 4.** *Conolophus marthae* sp. nov. Adult male with brand number 118 and PIT number 091-062-369, held by A. Jaramillo, field assistant of the Charles Darwin Research Station. This individual is not the Holotype.



**FIGURE 5.** *Conolophus marthae* sp. nov. Adult male with brand number 36 and PIT number 091-336-546. Details of the head. This individual is not the Holotype.



**FIGURE 6.** Head-bob display of *Conolophus marthae* sp. nov. Multiple series (a, b, c, and d) of head-bob performed by the Holotype (June 2006). The series were performed with the following time intervals between each other: a-b, 27 seconds; b-c, 34 seconds; c-d, 11 seconds.

**Coloration:** pinkish head, pinkish and black (dark) body and legs, with a black-striped pattern on the mid to posterior dorsal body. On both sides, five vertical black stripes occur between forelimb and hind limb,

along the dorsal-ventral axis. The first stripe is interrupted. Stripes 2–5 are joined horizontally, describing a complex pattern. Stripes are present but less evident on the ventral body. Dark tail.

**Behavioral characteristics:** The head-bob display (nodding behavior) consists of repeated modules. Each module comprises three series of multiple head movements (“ups and downs”; Fig. 6a–c) and is completely executed within a time interval of 4–5 seconds. Frequency of movements performed in each series is high, with 4 to 6 movements per second. Two sub-series, separated by a few deciseconds, may be recognized within series 2. A fourth, additional series, similar to series 3, may be observed occasionally (Fig. 6d).

**Etymology.** The new species is named in memory of Martha Rebecca Gentile, second daughter of the first author. Martha prematurely left this world. She was born dead, as consequence of a medical doctor’s negligence, on August 20<sup>th</sup> 2003.

**Distribution.** Thus far, this species is known to occur only on Volcan Wolf (Fig. 1), the northernmost volcano of Isla Isabela (Galápagos National Park, Ecuador).

**Remarks.** The new species is easily distinguished from the other two congeneric species. The color pattern is typical of the new species and was never observed in any of the populations of the other two named species. The origin and the nature of the pink pigmentation deserve further investigation. Nevertheless, it is instructive to note that when we surgically removed one pink scale, blood flowed out of the tissue of the removed scale, which immediately lost its pink color.

Traits i) and ii) in the diagnosis are more evident in males, whereas they are variable and generally less pronounced in females. Although in the Plaza Sur population of *C. subcristatus* almost flat dorsal head scales may be observed, such a trait never co-occurs in combination with the other traits characteristic of *C. marthae* sp. nov.

Although the “head-bob” pattern is slightly different between *C. subcristatus* populations in different islands (Gentile, unpublished data), the nodding behavior of *C. marthae* sp. nov. is very distinctive and characteristic. This is particularly relevant since it is exhibited in sympatry (syntopy) with *C. subcristatus*. None of the other species of land iguanas or any marine iguanas show a similar pattern (see Carpenter, 1982, for a comparison).

*Conolophus marthae* sp. nov. is distinct from the other two congeners by about 7% mtDNA genetic divergence, much higher than genetic divergence between *C. pallidus* and *C. subcristatus* (less than 2%, Gentile *et al.* 2009). Twenty-four nucleotide sites of the control region and seventy-two nucleotide sites in *cytb* gene sequences are diagnostically different and allow distinguishing between the new species and the other congeneric ones. The deep divergence is estimated to have started in a period when the Galápagos did not have their current configuration (Gentile *et al.* 2009). The absence of alleles shared with the other two species at the microsatellite locus CS7 and the presence of several private alleles at other loci (Tzika *et al.*, 2008; Gentile *et al.*, 2009) indicate genetic isolation, even with the syntopic population of *C. subcristatus*.

Occasional hybridization between marine (*Amblyrhynchus cristatus* Bell, 1825) and land iguanas (*C. subcristatus*) may still occur on Isla Plaza Sur, generating a black, brow-striped F1 hybrid (Rassmann *et al.*, 1997). *Conolophus marthae* sp. nov. lacks in any of the adaptive traits exhibited by marine iguanas (shortened snout; laterally compressed tail; developed caudal crest; long, recurved claws) and genetic data (Gentile *et al.* 2009) provide strong evidence that *C. marthae* sp. nov. did not originate by hybridization between marine and land (yellow) iguanas. A total of 120 individuals of *Conolophus marthae* sp. nov. were observed and sampled in three field trips, in 2005, 2006, and 2009 (see the paragraph “Notes added in proofs”).

Besides the taxonomic implications, *C. marthae* sp. nov. is very important as it is the only evidence of deep divergence within the Galápagos land iguana lineage. In fact, the new species carries an ancient evolutionary legacy, being the only remnant of a lineage originated when the Galápagos archipelago did not have its present configuration. *Conolophus marthae* sp. nov. is a narrow endemism and its population size is small. Its inclusion in the Red List of the International Union for Conservation of Nature (IUCN) as “critically endangered” has been recommended (Gentile *et al.* 2009).

## Acknowledgments

This work is in the frame of a conservation program in tight partnership between the University “Tor Vergata” and the Galápagos National Park. We gratefully thank the Galápagos National Park for giving us the permission to formally describe this new species and for agreeing in the choice of the nomen. We are deeply indebted with C. Marquez who is a crucial member of our team of investigation. For his dedication to Galápagos iguanas, he deserves a special mention here. We also thank M. Cruz, A. Fabiani, A. Jaramillo, X. Jaramillo, R. Palozzi, H. M. Snell, W. Tapia who contributed in sampling animals and collecting data. We express gratitude to V. Cedeño, V. Sbordoni, and the Charles Darwin Research Station for their support to this investigation.

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## Notes added in proofs

Recent publications debated whether nomenclatural availability of nomina of new species should always require the deposition of preserved specimens in collections (Dubois and Nemésio, 2007; Donegan, 2008; Nemésio, 2009). In particular, the issue of the Galápagos pink land iguanas has been specifically addressed by Nemésio (2009). We recommend extreme caution in the case of these iguanas prompted by concerns for the conservation of this newly discovered and obviously rare animal. The area inhabited by pink iguanas is limited to about 25 km<sup>2</sup> and based on extensive searches during three prolonged visits the adult population currently appears unlikely to exceed 200 animals. Here we designate a living Holotype permanently branded (n. 117) and marked with the Passive Integrated Transponder (PIT n. 091-601-303) inhabiting the area described. These redundant, permanent identification marks will insure identification of the Holotype. The legal authority governing biological diversity of the Galápagos Islands, the Galápagos National Park Service, has agreed that if continued monitoring of the population of pink iguanas suggests that the population is increasing, the Holotype will be captured, moved to an existing captive facility at the Galápagos National Park Center, Puerto Ayora, Isla Santa Cruz; where it will be maintained until it dies. Upon the Holotype's natural death, it will be preserved and deposited in the Governmental Galápagos collection, maintained by the Charles Darwin Foundation, Puerto Ayora, Galápagos.